World Journal of Surgery and Surgical Research

Research Article
Published: 03 Jul, 2024

Targeted NGS Sequencing in Nodular Goiter and Papillary Thyroid Carcinoma Reveals Striking Similarities - A Comparison in a Single Center

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Abstract

Thyroid nodules affect more than 5% of the population. However, even when using fine needle biopsy, not all of the lesions can be classified as benign or malignant. The aim of the present study was to investigate the main somatic genomic alterations in patients with benign Nodular Goiter (NG) and Papillary Carcinoma (PTC) to compare the molecular profiles and determine the correlation with the main clinical characteristics of these patients.

Materials and Methods: The study included 27 patients with nodular euthyroid goiters and 27 patients with papillary thyroid carcinoma treated at the Endocrine Surgery Clinic of the USBALE "Acad. Iv. Penchev". Tissue samples were obtained intraoperatively, and DNA was isolated from all tissues. Genetic analysis was performed by Next-Generation Sequencing (NGS) of all the samples *via* the Ion PGM™ tool.

Results: Among the 27 patients with PTC, 77 somatic mutations were found in 36 genes. The gene with the most pathogenic mutations in this group was *BRAF* (37%), followed by *TP53* and *PTEN*. In the group of 27 patients with NG, we found 49 variants in 25 genes. The genes most frequently affected by pathogenic mutations in this cohort were *NRAS* (11.1%) and *BRAF*.

Conclusion: Current targeted NGS sequencing of PTC samples showed the role of mutations in key genes, such as *TP53*, *BRAF*, *KIT*, *ERBB4*, and *APC*. Our study revealed specific hot spot somatic mutations in cancer genes, such as *TP53*, *BRAF*, *NRAS*, *APC*, *PTEN*, *STK11* and *ABL1*, in patients with NG. A somewhat unexpected finding that requires additional in-depth studies is the presence of *TP53* mutations in NG samples. Spot mutation testing revealed pathogenic mutations in both benign thyroid nodules and PTCs. However, more comprehensive NGS will be needed to better understand the role of specific driver mutations and to better distinguish between NG and PTC.

Keywords: Differentiated thyroid carcinoma; Nodular goiter; Somatic mutations; Next-Generation Sequencing (NGS)

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Received Date: *02 Jun 2024*
Accepted Date: *27 Jun 2024*
Published Date: *03 Jul 2024*

Citation:
Vidinov K, Dodova R, Petkova V, Kaneva R. Targeted NGS Sequencing in Nodular Goiter and Papillary Thyroid Carcinoma Reveals Striking Similarities - A Comparison in a Single Center. World J Surg Surgical Res. 2024; 7: 1558.

Introduction

The word goiter is used to describe a diffusely enlarged thyroid gland. However, when only palpation is used, thyroid nodules are found in approximately 5% of the population [1]. When conducting ultrasound screening, this percentage increases significantly. It can reach between 20% and 75% of the general population. A greater frequency of thyroid nodules is observed in iodine-deficient areas. Interestingly, thyroid nodules are rare in children, but their frequency increases with age. Women are affected 2 to 4 times more often than men are. Despite the high frequency of this disease, modern recommendations for conservative monitoring of benign thyroid nodules exist. Thyroid carcinoma is a rare malignancy in humans and accounts for up to 1% of cases. Papillary thyroid carcinoma is the most common endocrine cancer and presents clinically as a thyroid nodule [2]. It is found in approximately 5% of all thyroid nodules. It is characterized by a slow course and low aggressiveness. Early lymphatic and organ metastases appear in a small number of patients and significantly worsen the prognosis. This difference in the behavior of the two most common benign and malignant tumors of the thyroid gland determines the need for definitive diagnostic distinction. This is achieved to some extent thanks to Fine Needle Aspiration biopsy (FNA). The routine performance of FNA has changed the treatment of thyroid nodules by allowing us to avoid unnecessary operations on benign lesions and thus reducing the cost of treatment. On the other hand, preoperative FNA and proof of thyroid carcinoma suggest a one-stage operation–thyroidectomy–

in contrast to multiple operations in patients without preoperative cytological clarification. The sensitivity of FNA varies between 65% and 98%, and the specificity varies between 72% and 100%. Unfortunately, not all thyroid nodules can be classified correctly [3]. This gray area highlights the need to find additional methods for the preoperative differentiation of these diseases. Genetic testing could help to distinguish between benign nodules and thyroid cancer, and it is not surprising that in the latest revision of the Bethesda system, in addition to reconducting FNA and/or diagnostic lobectomy, genetic testing is also recommended. According to the 2015 American Thyroid Association (ATA) guidelines for adult patients with thyroid nodules and differentiated thyroid cancer, molecular testing may be used to supplement malignancy risk assessment for nodules of Atypia of Undetermined Significance (AUS)/Follicular Lesion of Undetermined Significance (FLUS) (Bethesda III), Follicular Neoplasm (FN)/Suspicious for a Follicular Neoplasm (SFN) (Bethesda IV) and Suspicious for Malignancy (SFM) (Bethesda V).

The international guidelines recommend that we perform molecular analysis only after undetermined cytological results are obtained. Some studies have shown that the presence of *BRAF* mutations in particular could be sufficient to determine the need for surgery [4]. According to European Thyroid Association (WHO), *BRAF*, RET/PTC mutation, PAX8/PPARG mutation and *RAS* mutation should be detected when there are cytologically indeterminate nodules [5]. The thyroid nodules in the gray area (Bethesda III and IV) may benefit more from *BRAF* mutation detection because thyroid follicular neoplasms with *BRAF* mutation are substantially less common [6].

Genetic testing of thyroid FNA samples could increase both the diagnostic accuracy and the prognosis. Treatment decisions should be made based on medical imaging, FNA histopathological and genetic data. No prior comprehensive molecular genetic analysis has been performed in Bulgarian patients with thyroid pathology.

Therefore, the aim of the current study was to analyze somatic genomic alterations in hot spots using a panel of 50 cancer-related genes in 27 patients with benign nodular goiter and 27 patients with papillary carcinomas and to compare the obtained genetic profiles with their main clinicopathological characteristics.

Materials and Methods

The study included 27 patients with nodular euthyroid goiters and 27 patients with papillary thyroid carcinoma treated at the Endocrine Surgery Clinic of the USBALE "Acad. Iv. Penchev" for the period January 2017 to December 2021 (Table 1, 2).

All patients provided written informed consent to participate in this study. The study protocol was reviewed and approved by the ethics committee of USBALE Acad. Iv. Penchev. The patients were analyzed according to the following clinical features: 1) sex; 2) type of hereditary pathology of the thyroid gland (malignant or benign); 3) histology of the cancer; 4) PTC subtype; 5) time of diagnosis (age of the patient); 4) the spread of the tumor in the gland (multifocality) for malignant disease; 6) the size of the tumor; 7) the spread of the tumor in the lymph nodes (lymphatic metastases); 8) the presence of distant metastases; and 9) the stage of the disease.

DNA was isolated from fresh-frozen samples taken during surgery. Half of the tumor was transferred to -80°C for preservation, and the other half was sent for histopathological diagnosis. DNA was isolated from all tissues with the QIAamp DNA Mini *KIT* in accordance with the manufacturer's recommendations, with subsequent molecular genetic analysis for mutation hotspot variants in genes associated with tumor development (Table 3). Molecular genetic analysis was performed on the next-generation sequencing platform Ion PGM™ System using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific), and the data were analyzed with the Torrent Suite Version 5.18 (Thermo Fisher Scientific) and VarSeq (Golden Helix) bioinformatic programs. The evaluation of the clinical effect of the identified variants was performed based on the ClinVar, VarSome, Franklin and Cosmic databases. Preoperatively, the patients underwent Ultrasound (US) of the neck, and a qualitative assessment of their nodules was performed by a board-certified endocrinologist. Ultrasonography was used to determine the size, location, and multicentricity of the carcinomas and lymph node

Table 1: Distribution by age, sex and histological variant of the 27 cases with thyroid cancer.

Gender	Number of patients (n)	Percentage (%)					
Male	3	11.2					
Female	24	88.8					
Total	27	100					
Age	**Number**	**Percentage (%)**					
Below 45 years	12	44.4					
Above 45 years	15	55.6					
Total	27	100					
Histological subtype of Papillary thyroid cancer	**n**	**%**	**Multifocal tumors**	**Stage**			
				I	**II**	**III**	**IV**
Classical variant	24	88.8	4	24	0	2	0
Follicular variant	3	11.2	1	1	0	0	0
Total	27	100	5	25	0	2	0
Close relatives with thyroid disease	**n**	**Percentage (%)**					
With malignant disease	0	0					
With benign disease	12	44.4					
Without any thyroid disease	15	55.6					

metastases. The final diagnosis of all patients was established by histopathology. Thyroid cancer was staged according to the eighth edition of the American Joint Committee on Cancer (AJCC) TNM classification for differentiated thyroid carcinoma. The data were analyzed and interpreted with the SPSS 26.0 software package (SPSS, Inc., Chicago, IL). The difference between groups was analyzed with the Chi-square test and Fisher's exact test, with $p<0.05$ considered to indicate statistical significance.

Results

Among the 27 patients with papillary thyroid carcinoma studied, 77 had mutations in 36 genes (Figure 1). A full list of the detected PTC variants is provided in Supplementary Table 1.

When compared to clinical data, we found that 9 variants were classified as pathogenic, 15 variants as likely pathogenic, 13 variants of unclear significance, 9 variants as probably benign, and 30 as benign.

The most frequently affected gene with pathogenic mutations in this group was *BRAF* (10 patients - 37%), followed by *TP53* (9 patients - 33.3%) and *PTEN* (3 patients - 11.1%). Among the likely pathogenic mutations, the most affected gene was *APC* (4/27, 14.8%), followed by *ERBB4* (3/27, 11.1%) and *CDKN2A* (3/27, 11.1%). The genes most frequently affected by mutations of unclear significance were *FGFR3* (4/27, 14.8%) and *KIT* (3/27, 11.1%). Among the benign mutations, *KDR* (15/27 - 55.5%) and *KIT* (9/27 - 33.3.5%) were most common. Interestingly, we had no patients without somatic mutations. The most frequently mutated gene in this group was *TP53*, which had 8 different somatic mutations-5 pathological mutations, 1 likely pathogenic mutation and 2 benign mutations.

The combination of the three most common pathogenic mutations (in *BRAF*, *TP53*, and *PTEN*) was found in one patient–a woman (39 years old) with pT1N0M0. Her mother and sister had Hashimoto thyroiditis. Pathogenic mutations in the *BRAF* and *TP53* genes occurred in 3 patients–the 1 patient already described, one male (59 years) with pT3N0M0 and one female (37 years) with pT1N1M0. Of these, only the 37-year-old woman reported having thyroid disease (nodular goiter) as a direct relative (mother).

In the two patients with third-stage disease, we found a combination of the different types of mutations. The first patient was a male described in our study with a combination of the two most common pathogenic mutations; however, mutations with a possibly pathogenic nature in *ERBB4*, *CDKN2A*, *KIT* and *TP53*; variants of unclear clinical significance in *NOTCH1* and *PTEN*; and variants of benign nature in *ATM*, *FGFR3*, *CDKN2A*, and *KIT*. The second patient was a 68-year-old woman with pathogenic mutations in *BRAF* and benign mutations in the *ATM*, *VHL*, *APC*, *FGFR*, *HRAS*, *KDR*, *KIT*, *PDGFRA*, and *TP53* genes. There was evidence of nodular goiter in her family, mother and sister.

Table 2: Distribution by age, sex and histological variant of the 27 cases with nodular goiter.

Gender	Number of patients (n)	Percentage (%)
Male	5	18.5
Female	22	81.5
Total	27	100
Age	**n**	**Percentage (%)**
Below 45 years	10	37.1
Above 45 years	17	62.9
Total	24	100
Close relatives with thyroid disease	**n**	**Percentage (%)**
With malignant disease	1	3.7
With benign disease	6	22.2
Without any thyroid disease	20	74.07

Table 3: Genes included in Ion AmpliSeq™ Cancer Hotspot Panel v2.

ABL1	EGFR	GNAS	KRAS	PTPN11
AKT1	ERBB2	GNAQ	MET	RB1
ALK	ERBB4	HNF1A	MLH1	RET
APC	EZH2	HRAS	MPL	SMAD4
ATM	FBXW7	IDH1	NOTCH1	SMARCB1
BRAF	FGFR1	JAK2	NPM1	SMO
CDH1	FGFR2	JAK3	NRAS	SRC
CDKN2A	FGFR3	IDH2	PDGFRA	STK11
CSF1R	FLT3	KDR	PIK3CA	TP53
CTNNB1	GNA11	KIT	PTEN	VHL

According to the results of the statistical analysis with the Chi-square test, there was a significant relationship ($p=0.05$) between the presence of a pathogenic mutation in *TP53* and unilateral lymph node involvement in papillary carcinoma. Given the small number of patients, we applied the "Fisher's exact test" method ($p=0.115$) without statistical significance. We found a relationship between *TP53* mutation and lymph node involvement in the central compartment (Chi-square test, $p=0.06$; Fisher's exact test, $p=0.25$). For the remaining pathogenic mutations and clinical features, such as multifocality, lymphatic metastasis and heredity, no statistically significant relationships were established.

For likely pathogenic mutations and Variants with Unclear Clinical Significance (VUS), we found no statistically significant relationship with patient clinical characteristics, such as multifocality, lymph node engagement and heredity.

Among the 27 patients with nodular euthyroid goiters studied, 49 variants with mutations in 25 genes were found (Figure 2). A full list of the detected variants in nodular goiters is provided in Supplementary Table 2.

According to the clinical database, 6 variants were classified as pathogenic, 12 variants as likely pathogenic, 9 variants of unclear significance, and 22 variants as benign. The most frequently affected genes in this cohort were *NRAS* (3/27, 11.1%) and *BRAF* (2/27, 7.4%). Among the likely pathogenic mutations, the most frequent were *APC* (2/27-7.4%), RB1 (2/27-7.4%) and *ERBB4* (2/27-7.4%). The genes most frequently affected by mutations of unclear significance were *NPM1* (3/27, 11.1%) and *TP53* (2/27, 7.4%). Benign mutations, were most often found in *KDR* (11/27-40.7%), *APC* (6/27-22.2%), *ERBB4* (6/27-22.2%) and *TP53* (5/27-18.5%).

In one patient, NS019, 2 pathogenic mutations were found in *BRAF* and *NRAS*. The patient was a female and had involvement of only one thyroid lobe, and the maximum diameter of the formations in the thyroid gland was 2 cm. There was no evidence of thyroid disorders in her family. In NS036, two mutations in *BRAF* and *TP53* were found. In another subgroup of NG patients, 2 pathogenic frameshift mutations were found: NS049 (in *NPM1* and *FGFR3*),

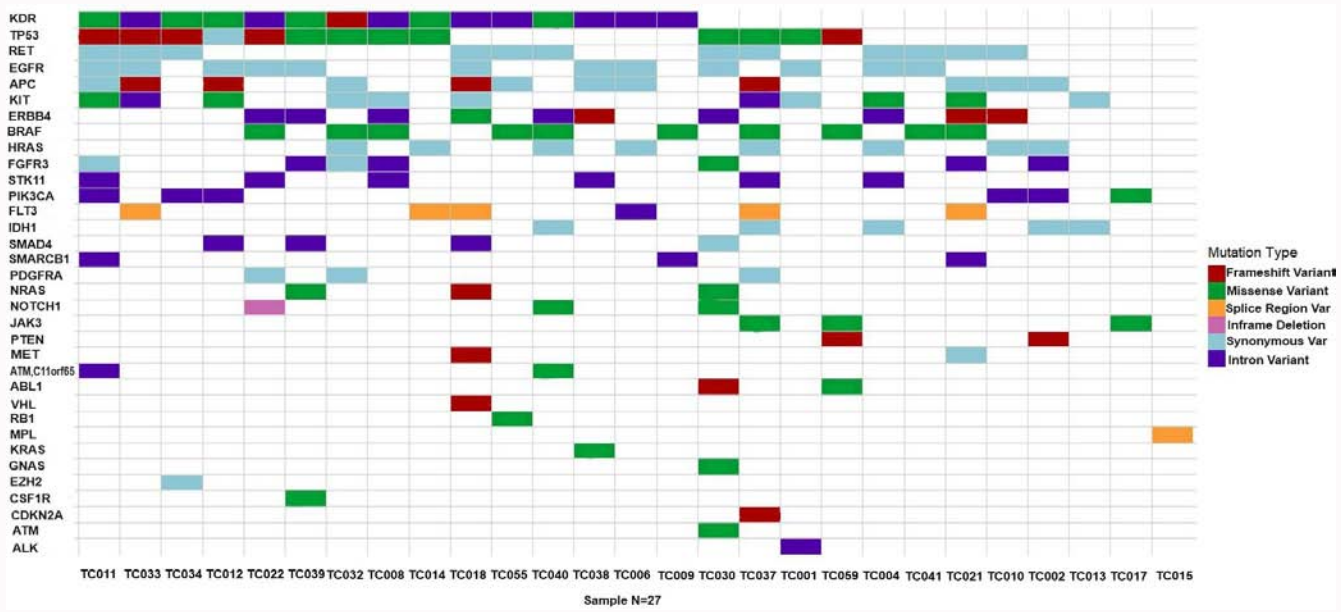

Figure 1: Distribution of somatic mutations in the studied genes from the panel found in the 27 patients with papillary thyroid cancer.
Legend: Type of mutations is shown in different colors; frameshift – red; missense – dark green; splice site variants – orange; inframe deletions – pink; synonymous- blue, intron variants –purple.

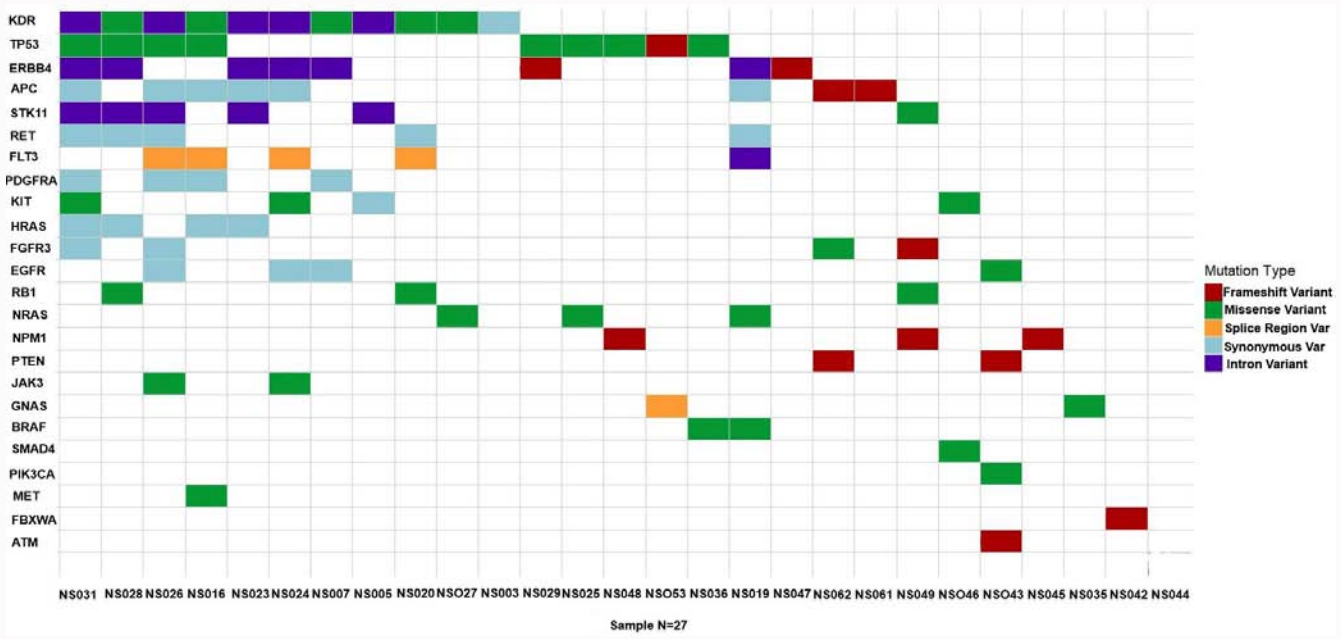

Figure 2: Distribution of somatic mutations in the studied genes from the panel found in the 27 patients with nodular goiter.
Legend: Type of mutations is shown in different colors; frameshift – red; missense – dark green; splice site variants – orange; synonymous- blue; intron variants –purple; no variants – gray

NS062 (in *PTEN* and *APC*) and NN043 (in *ATM* and *PTEN*) (Figure 2).

According to the statistical analysis of pathogenic variants of somatic mutations using the Chi-square method, we found a statistically significant association between the intrathoracic location of the thyroid pathology and the *BRAF* gene (p=0.024), but the "Fisher's exact test" method did not confirm statistical significance (p=0.08). For the remaining somatic pathological mutations, we found no statistically significant relationship with patient clinical characteristics.

For mutations of Unclear Significance (VUS), likely pathogenic or benign, we did not find statistical significance with any of the set criteria.

Discussion

Most of the studies on this topic show that the main causes of thyroid cancer development are gene mutations, translocations and amplifications, gene methylation disturbances and microRNA

dysregulation [7,8]. Our modern understanding of this disease is that the cornerstone of carcinogenesis is the inactivation of suppressor genes and the activation of oncogenes [9,10]. Mutations observed in papillary thyroid carcinoma commonly affect the *RAS*, *BRAF*, *PTEN*, *CTNNB1*, *TP53*, *IDH1*, *ALK* and *EGFR* genes [11-13].

The *BRAF* gene plays a special role in PTC [14,15]. The most common *BRAF* gene mutation reported in PTC is the transversion of thymine to adenine at position 1799 (T1799A) in exon 15, resulting in the substitution of Valine (V) with glutamic acid (E) at codon 600 (V600E) [10,16,17]. The V600E mutation accounts for 99% of all *BRAF* mutations found in thyroid cancer [18]. In our study, the frequency of this mutation was 37%, which is comparable to the lower end of the range of frequencies published by other authors [7,19,20]. It occurs significantly more often in PTCs than in NGs (found only in two patients). Pathogenic mutations were the most common mutations in the thyroid cancer group, followed by those in the nodular goiter group. Unfortunately, due to the small sample size, we did not find a statistically significant relationship between the mutation and the individual clinical signs of the disease. It is still interesting to note the association of the mutation with the intrathoracic location in patients with nodular goiters. However, it is just of suggestive statistical significance, probably due to the small sample size.

The second most common mutation is in the *RAS* proto-oncogene. *RAS*s play an important role in the initiation of thyrocytic neoplastic transformation [21,22]. *RAS* family (*N-RAS*, *H-RAS*, *K-RAS*) proto-oncogenes are located on chromosomes 1, 11, and 12, respectively. This family plays an important role in cell growth and differentiation. They encode G membrane proteins that exhibit intrinsic GTPase activity and are involved in signal transduction from the membrane tyrosine kinase receptor to the cell nucleus. Changes in codons 12, 13 and 61 result in active oncogenes [23]. *RAS* mutations can be detected in both benign and malignant thyroid nodular diseases [24,25]. These mutations can be detected in approximately 50% of patients with FTC, in 20% of patients with PTC, in no more than 40% of poorly differentiated and anaplastic patients, and in approximately 30% of follicular adenomas [26]. In our study, based on the *RAS* mutational profile, we could clearly distinguish the two groups of diseases. We have a single patient with a mutation in the *RAS* gene in papillary carcinoma, while in nodular goiter; the pathogenic mutation in *NRAS* is the most common (3/27-11.1%). However, statistical analysis revealed no associations between clinical features and *RAS* mutations. Interestingly, *NRAS* was previously found in two benign histological samples [27] and in a single nodular goiter in a Chinese patient [28]. However, recent studies suggest that *RAS* mutation-positive benign nodules demand conservative treatment [29]. To evaluate more precisely the clinical impact of *RAS* mutations in thyroid malignancies, we should consider the whole molecular profile of mutations and genomic abnormalities.

By its very nature, the Tumor Protein p53 (*TP53*) is a gatekeeper [30]. *TP53* encodes the protein p53, which is known as the "guardian of the genome". *TP53* mutations are frequently observed in ATC (70%-80%) [31,32]. *CTNNB1* and *TP53* mutations are mostly found in Anaplastic (ATC) or Poorly Differentiated Thyroid Carcinoma (PDTC) patients. For this reason, most authors believe that these cells are directly linked to cell dedifferentiation or a late event in the development of follicular cell-derived cancers [33,34]. Interestingly, in our study, we found mutations in *TP53* both in NGs and in PTCs. In the papillary thyroid carcinoma cohort, a pathogenic mutation was found in 6 patients (22.2%), while in only 1 patient, it was found in a nodular goiter (3.7%). Furthermore, in patients with PTC, pathogenic mutations are more common, while in patients with NG, variations of unclear significance and benign changes are found. Notably, unlike *RAS* mutations, which occur in carcinomas with a relatively benign course, cancers in patients with *TP53* or *TERT* mutations, are associated with more aggressive TC and higher recurrence and mortality risks [35].

In our study, there was a strong statistical relationship between *TP53* mutation and lymph node involvement in the central compartment (Chi-square test, p=0.06; Fisher's exact test, p=0.25). The results obtained by our study support an association with more aggressive behavior, but analysis of a larger sample is warranted.

Interestingly, in the nodular goiter group, there was a recurrent mutation in the *NPM1* gene, which was found in three patients. The gene encodes the protein Nucleophosmin (*NPM1*), which is involved in mRNA transport, chromatin remodeling, apoptosis and genome stability. *NPM1* is commonly overexpressed in different cancers, including thyroid cancer, and is mutated, rearranged or sporadically deleted [36]. The mutation is new likely pathogenic and not present in the public databases, but located in a region where a mutation common in AML is found. Mutations in the *NPM1* gene are detected in one-third of adult Acute Myeloid Leukemia (AML) patients, and 50% to 60% of AML patients with a normal karyotype and different therapies targeting the *NPM1* protein are being developed [37].

Another interesting finding in patients with both PTC and nodular goiter is the comparatively high frequency of missense variations in genes such as *KDR* (Q472H in 4 NG and 6 PTC cases and S266L in one NG, in 1 PTC case); *TP53* (P72R in 5 NG and 3 additional missense variants, as well as in 3 PTC cases) (Figure 1, 2); and *KIT* (M541L in 3 NG and 2 PTC and additional variant I563K in one NG case).

According to a study of 25 Polish patients with a "gray area" cytology, the most common mutations were in *KDR*, *TP53* and *RET*. The samples were also subjected to NGS, similar to the methods used in the present study. *KDR* mutations were found in 3 of the malignant samples compared to the benign samples [38].

In previous studies, the *KDR* Q472H mutation has been linked to primary *EGFR* TKI resistance in lung cancer [39]. The *KDR* gene encodes *VEGFR-2*. Therefore, patients with the *KDR* Q472H mutation were found to have elevated VEGFA levels and increased tumor microvessel density [40].

Owing to increasingly widespread testing with NGS in patients with confirmed *EGFR* TKI resistance in Non-Small Cell Lung Cancer (NSCLC), the nondriver mutations *TP53* P72R, *KDR* Q472H, and *KIT* M541L, beyond T790M, were found to be directly linked to primary resistance [39].

Different activating mutations in the MAPK-ERK and PI3K-AKT-mTOR cascades are important steps in thyroid carcinogenesis. The alterations occurring in the MAPK pathway are characterized by unique clinicopathological characteristics, gene expression, and DNA methylation profiles in TC [41]. According to the current guidelines, treatment with a kinase inhibitor is recommended for patients with differentiated thyroid cancer who are Resistant to Radiation Therapy (RAI-R). These patients are usually those with PTC who have metastatic, rapidly progressive, symptomatic, and/or imminently

threatening disease that is not sensitive to local control *via* other approaches. Clinicians should be careful when managing these patients because of their tumor mutation profiles and characteristics, as well as their symptoms and treatment-related side effects [42,43].

Common polymorphic variants in the *FLT3* gene possibly affecting splicing were found in both the PTC and NG patients. *FLT3* encodes FMS-related tyrosine kinase 3, a receptor that regulates hematopoietic progenitor and dendritic cells. The rs2491231 variant (c.1310-3T>C) was found in 5 PTCs and 4 NGs, and the rs75580865 variant (c.2053+23A>G) was found in one PTC sample and 1 NG sample. A high frequency of mutations in the *FLT3* gene was reported in follicular thyroid cancer patients in the Polish population [44]. In addition, in the population database GnomAD, the variant minor allele varies between 0.29 and 0.38.

A recent GWAS study of large sample of Island and UK Bio bank revealed a rare *FLT3* variant rs76428106 was linked to an increased risk of autoimmune thyroid disease. Through RNA sequencing, it was shown that the rs76428106 C allele generates a cryptic splice site that introduces a stop codon in 30% of transcripts that are predicted to encode a truncated protein that lacks its tyrosine kinase domains. The frequency of each copy of rs76428106-C doubles the plasma level of the FTL3 ligand. Activating somatic mutations in *FLT3* are associated with acute myeloid leukemia [8] with a poor prognosis, and rs76428106-C also predisposes individuals to acute myeloid leukemia [45].

Nonetheless, our study has several limitations, related to the small sample size, as well as the analysis of only hot spot mutations but not the complete coding sequencing of the genes from the panel. It focuses only on somatic mutations detected at the DNA level, point mutations and indels but does not include gene fusions or large deletions/amplifications or on some genes, such as TERT, mutations, which have been proven to be of prognostic relevance.

Due most likely to the small sample size, it was not possible to establish significant correlations between particular clinical characteristics and hotspot mutations. Nevertheless, genetic profiling data for nodular goiters are not abundant in the literature, and our results already indicate a tendency toward a comparatively high frequency of *TP53* mutations or other driver genes, such as *APC*, *PTEN*, *STK11* and *RB1*, in nodular goiters. The multistep theory for cancer development involving the critical role of several guardians of the genome, such as *TP53*, *APC*, *ATM*, and other driver genes, such as the *BRAF* and *RAS* genes, was clearly demonstrated. However, more comprehensive profiling is needed to better differentiate between benign nodules and cancer. NGS in combination with RNA sequencing to detect fusions will improve the ability to define common mutation signatures and allow the design of a specific targeted panel for diagnostic purposes with high sensitivity and specificity. The presence of specific molecular profiles may be used for determining patient prognosis and for guiding the management of nodules with indeterminate cytology.

Conclusion

Current targeted NGS sequencing of PTC samples showed the role of mutations in key genes - *BRAF*, *TP53*, *PTEN*, *APC*, *ERBB4* and *CDKN2A*. Our study revealed specific hotspot somatic mutations in cancer genes, such as *NRAS*, *BRAF*, *APC*, RB1 and *ERBB4*, in nodular goiters. In patients with combinations of several pathogenic mutations detected by hotspot panel sequencing, careful follow-up is warranted. However, more comprehensive mutation profiling is needed to better differentiate between benign nodules and thyroid cancer.

Funding

This study received funding from one Research Grant, KP-06-H23/9/18.12.2018 Ministry of Education and Science, Bulgaria.

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